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Maltreatment and Epigenetic Aging in Infants – A DNA Methylation Analysis

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2010

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An abstract of
a thesis submitted to the Faculty of the
Rollins School of Public Health of Emory University
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Master of Public Health
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2022

Abstract

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By Jay Visbal

Background: Research into the relationship between trauma and future health outcomes is being explored across a wide range of biological pathways. Studies have shown that increasing divergence between epigenetic age and chronological age in adults is associated with age-related disease outcomes in adults. In adolescents, certain types of trauma have been shown to be associated with increased epigenetic aging. We wanted to better understand whether early life exposure to maltreatment could result in a similar increase in epigenetic aging in pediatric populations as it does in adult and adolescent populations.

Methods: We explored whether physical trauma or a context of psychosocial adversity are associated with increased epigenetic aging in 65 pediatric patients brought to Emergency rooms with physical injuries. DNAm levels were measured in buccal epithelial cells using the skin-blood and PedBE epigenetic clocks to estimate epigenetic age. We regressed these epigenetic age predictions on chronological age. Age acceleration was defined as the residuals extracted from this linear model. Increased deviation from the predicted value of the model indicated increasing epigenetic age acceleration.

Results and Discussion:
Our results suggest that intentionally abusive injury and psychosocial risk factors do not correlate with increased epigenetic aging. However, using the PedBE clock, fatal physical injury was found to be associated with increased epigenetic aging. These preliminary results are contrary to prior research which may be due to the very early age of the participants or the small size of the study population. Nonetheless, the broader epigenetic ageing process may be one of the many biological pathways perturbed by exposure to physical or emotional maltreatment. And this process may be observable very rapidly after initial exposure.

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INTRODUCTION

Epidemiology of Child Abuse and Psychosocial Adversity. Child abuse and neglect is a major public health issue. In the United States in 2018, there were an estimated 678,000 victims of child abuse and neglect with 1,770 children fatally injured from maltreatment; a rate of 2.39 per 100,000 children.[1] One meta-analysis estimated that worldwide, at least 44% of children in high-income and 59% of children in low-and-middle income countries had been subjected to some type of physical, emotional, or sexual violence; or had witnessed domestic or community violence in the past year.[2] Younger children are particularly vulnerable, with 46% of deaths occurring in children less than one year old. In the Child Abuse Prevention and Treatment Act of 1974 (CAPTA, P.L. 93-247) child maltreatment is defined as a caretaker acting or failing to act in a way that results in death, serious physical or emotional harm, sexual abuse, or exploitation of a child. From 2014 to 2018, there was an 8.4% increase in the number of children who received child protective services investigations (3.26 million to 3.53 million). Of the maltreatment reported in 2018, 61% of children were neglected, 11% were physically abused, and 7% were sexually abused while 16% were found to be victims of more than one type of maltreatment.[1]

It is difficult to capture the full picture of child abuse and neglect since there are differences in reporting across state protective services. When states change their reporting procedures or create alternative mediation pathways, it can dramatically affect national numbers reported, therefore statistical trends should be viewed carefully.[1] Additionally, unrecognized child abuse is prevalent. In a hospital analysis of children with abusive head trauma, 31.2% were misdiagnosed and 80% of deaths in the misdiagnosed group may have been averted by earlier abuse diagnosis.[3] Abuse recognition remains a complicated charge for practitioners. It is highly

likely that child maltreatment is underreported because of state procedural differences, misdiagnosis, social stigma, and the vulnerability of young children; thus, the public health burden is presumably greater than what is documented.[4]

There are numerous risk factors for child maltreatment. The risk of trauma is higher in children born prematurely or with several medical conditions.[5] Perpetrators of maltreatment are more likely to have depression, a history of suicide, major life stressors, been in foster care, or abandoned as a child. Other risk factors include unwanted pregnancy and increased number of separations from the child in the first year. In one study, researchers found that 97% of child abuse cases studied revealed at least one or more of the following conditions present in the caretakers: alcohol or drug abuse, psychiatric disorder, a history of violence, or a jail sentence.[6]

Extreme physical injury from maltreatment does not typically occur in a vacuum, but often co-occurs with other potential stressors in a child's young life. Physical trauma can be a marker for a range of psychosocial adversity such as medical neglect, emotional neglect, verbal abuse, or living with a caretaker with mental illness or substance use disorder. Collectively, these factors form critical domains of the environment in which early development occurs.[7] This environment of adversity can have wide ranging impacts on children's health and development, in addition to the abusive trauma itself. Researchers have found that the acute psychosocial damage done to children's neurological, cognitive, and emotional development within this environment tends to have a greater impact on future well-being than the physical injury of abuse.[8,9]

Later Health Outcomes Associated with Abuse/Psychosocial Adversity. For children who survive early childhood abuse and psychosocial adversity there are a wide range of

potential long-term adverse health consequences including obesity, malnutrition, smoking, depressive disorder, anxiety, eating disorders, PTSD, drug use, cigarette use, and suicidal ideation.[10,11] Adolescents who have been victims of physical abuse were shown to have higher body mass indexes than their unexposed peers, even when controlling for socio-demographic, behavioral, and psychological confounders.[12]

Early life adversity is linked to marked alteration in brain structure and function, especially in stress sensitive areas such as the hippocampus and amygdala; both important for learning and emotion regulation, as well as the pre-frontal cortex; key in executive functioning and higher cognition.[13-15] Additionally, adverse childhood experiences are known to contribute to pro-inflammatory pathways in adulthood.[16] However, the precise biopathways between early life maltreatment and later-life disease remain unknown for several reasons. Typically, there is a significant time delay between child maltreatment and adverse health outcomes. This time delay may allow confounding exposures and interactions to muddy the causal pathway. Determining causality amongst social complexity without the power of randomized controlled trials, can be especially difficult.[17] Additionally, many studies of child maltreatment rely on retrospective self-reporting which may differ from prospective assessment.

Epigenetics and Epigenetic Age. Biological embedding is the process where environmental exposures and experiences lead to durable change in physiological and developmental states.[18] Early development is an especially delicate period for biological embedding because of increased sensitivity to perturbation and frenetic growth.[17] Biological embedding and long-term changes in gene expression may occur through epigenetic modifications, specifically alterations in DNA methyl groups or histone acetylation.[19] DNA

methylation (DNAm) is measured predominantly where cytosine and guanine are separated by a phosphate group within the DNA helix (CpG site).[20] CpG sites are important regulators of gene expression, which can be altered by the presence of a methyl group on the cytosine.[20] These alterations in DNAm and subsequent gene expression can result in functional changes to cells, tissues, and organs, ultimately leading to different phenotypes.[21] Several studies have shown an association between childhood adversity and both widespread genome methylation alteration as well as gene-specific effects on dopamine, serotonin, and glutamate; all important molecules for mental health and neural signaling.[17]

Biological embedding of DNAm changes are now being studied as potential markers for future health outcomes. Early developmental adversity has also been associated with DNAm modifications that may be related to psychiatric risk, where adversity before the age of 3 years appeared to be most impactful.[22] Additionally, researchers have studied telomere erosion, a marker of biological aging, and its association with developmental trauma as well as epigenetic changes associated with chronic stress.[23,24] Thus, DNAm, and possibly biological aging, may be a subclinical link between early life trauma and adverse health outcomes later in life.

Biomarkers of ageing measured via DNAm at hundreds of CpG sites reveal underlying maintenance and development processes of biological aging that correlate with chronological age.[25] Differences between DNAm age and chronological age, known as accelerated DNAm aging, are associated with age-related health outcomes in adults.[26] In adults, higher DNAm age acceleration is associated with an increase in mortality, cognitive decline, and a decrease in time until death.[25] These epigenetic aging processes can be perturbed by exterior stressors leading to accelerated DNAm aging and potentially to adverse health outcomes. Javonovic et al (2017) have shown that exposure to violence during development is associated with DNAm changes.[27]

Therefore, epigenetic age may be useful for learning about early life impacts of abuse and psychosocial adversity on children.

Research Gap Filled and Objectives of this Study. Taken together, child maltreatment is a prevalent and underreported public health issue. Physical abuse and psychosocial adversity are associated with a wide range of physical, mental, and emotional long-term health consequences. Epigenetic age acceleration may help explain an underlying mechanism involved in these relationships. To date, most research on the link between child maltreatment and accelerated aging has been conducted using retrospective survey methods with adults where there is a substantial lag between exposure and outcome. Of the three published studies investigating the impacts of adversity on epigenetic age, all were performed in adults.[28] Retrospective self- reporting of childhood adversity has the potential for misclassification and bias since prospective and retrospective measurement may disagree.[29] Participant temperament may bias retrospective adversity measures by underestimating the effect of adversity on objectively-measured health outcomes (e.g., biomarkers or neuropsychological tests) and overestimating the effect of adversity on self-reported outcomes.[29] Adults may choose not to share intimate details of childhood to avoid shame.[30] Or the presence of disease, psychopathology, or certain personality traits may unintentionally increase a participant's inclination to report childhood adversity.[31,32] These reporting errors and others may cloud the causal pathway in retrospective analyses performed in adulthood.

Additionally, epigenetic age acceleration is a durable, though not a permanent biological phenomenon. In retrospective studies with adults, epigenetic age acceleration is being measured many years after exposure to childhood adversity. The association of early life adversity with

epigenetic age acceleration may attenuate as natural ageing and additional perturbations mask the early developmental exposure of interest.[33] Or, it is possible that age acceleration accumulates after early life adversity, perhaps influenced by some of the other stressors and experiences associated with early adversity. These retrospective studies in adults may suffer from misclassification of the exposure and are not able to address whether impacts on biological aging are established proximal to adversity during early childhood or later on.

We aim to address this research gap by studying this relationship in very young children, cross-sectionally at time of diagnosis. Dunn et al studied the impacts of adversity at different life stages on DNAm and found that the largest effect on DNAm occurs before age three.[22] Since DNAm adapts over time, measuring childhood adversity 30-60 years after exposure has a smaller probability of observing an associated outcome. This analysis is more proximal to the exposure than previous studies and allows for a higher chance of detecting effects on DNAm. Additionally, earlier measurement removes the need to control for extra potential confounding variables. We aim to understand whether there is a relationship between physical abuse and psychosocial adversity during development and accelerated DNAm aging, measured in very young children (ages 0-4 years).

METHODS

Cohort. Children between zero and four years old with traumatic injury who visited the Pediatric Emergency Department at Ann & Robert H. Lurie Children's Hospital of Chicago and Cincinnati Children's Hospital were evaluated by the Child Abuse and Assessment Teams. Children meeting abuse criteria were enrolled prospectively, then an expert panel composed of a child abuse expert, injury biomechanics expert, and a pediatric emergency physician categorized

the traumatic injuries of the children as either abusive or accidental. Cheek swab buccal cells were collected for DNA extraction and DNAm measurement using the Illumina Infinium Methylation EPIC BeadChip. This study was reviewed and approved by both the Lurie Children's Hospital and Cincinnati Children's Hospital Institutional Review Boards (IRB). All data points have been de-identified. DNA methylation data were processed, and quality control steps were administered via standard protocols, such as the exclusion of poor performing samples and poorly detected probes, and data were normalized with functional normalization and beta mixture quantile normalization.

Defining Abusive versus Accidental Injury. Participants were characterized as physically abused or non-abused via a multi-step approach. Where there was exterior evidence to confirm a diagnosis of accident or abuse such as video at daycare of a fall, confession of abuse by the perpetrator, or injury that occurred in a public space, cases were considered documented. Cases without documentation were independently classified as abusive or accidental by a three-person expert panel consisting of a pediatric emergency physician, a child abuse expert, and an injury biomechanics expert. Expert panelists had all published on pediatric physical abuse and were recognized as leaders within the field of accurate diagnosis of physical abuse. Panelists were blinded to case data on psychosocial risk factors such as history of domestic violence, substance abuse, or criminal activity.[34]

Psychosocial Adversity. We created a cumulative risk score for psychosocial adversity, based on the presence of psychosocial risk factors (PRF) experienced by the child's caregivers. PRF scores ranged from 0-6, based on the presence of the following attributes: criminal history

of caregiver, prior child social service involvement, domestic violence in the home environment, caregivers verbalizing negative attributes of the child, substance abuse history, mental illness or anger management problems.[35] The score was the total count of PRFs present for each child. PRF score raters were blinded to the physical abuse vs. accidental injury documentation for each participant.

Measurement of DNA methylation and Epigenetic Age. Buccal epithelial cells were obtained from all participants for analysis. DNAm levels were measured at ~850K CpGs with the Illumina EPIC Array and were processed in R using the *minfi* package; probes with poor detection p-values (> 0.05) were excluded, data were corrected for dye bias, then normalized via functional normalization (FunNorm). We used the *sva* package to identify and estimate major sources of variation in the DNA methylation data which may represent unmeasured confounding. From these normalized data, 94 CpG sites were used with the Pediatric-Buccal-Epigenetic (PedBE) clock to estimate epigenetic age. For comparison, epigenetic age was also estimated via the skin-blood clock, via 391 CpG sites.[36] Using both clocks for comparison, we calculated age acceleration by regressing DNAm age on reported chronological age and extracted the residuals from these models.

Statistical Analysis. Linear regression analysis was conducted in R version 3.6. First, we examined associations between DNAm age (skin blood/PedBE) and reported age using correlations and scatterplots. We also examined whether proportions of epithelial cells, fibroblasts, and immune cells were associated with age acceleration as measured by either clock. Epigenetic age and age acceleration were incorporated as continuous dependent variables. Three

models were tested using independent variables: injury type (abuse vs. trauma), PRF score (0-6), and injury severity (mild, moderate, severe, fatal). Abuse or accidental trauma was included as a binary independent variable and PRF score was integrated as a six-level factor for the independent variable. Finally, injury severity was assessed as a four-level factor variable. We then tested for differences in age acceleration associated with abusive injury, higher psychosocial adversity score, and higher injury severity using the *mass* package. For all models we adjusted for insurance status and sex. White's sandwich estimator was used to estimate standard errors and p-values for robust regressions to protect against potential heteroscedasticity.

RESULTS

Skin blood and PedBE epigenetic clocks both had a statistically significant pearson correlation (rho = 0.94) with chronological ages (figures 1 and 2). We then regressed these epigenetic age predictions on chronological age. Age acceleration was defined as the residuals extracted from this linear model. Increased deviation from the predicted value of the model indicated increasing age acceleration. Age acceleration values clustered around zero for most samples, with only a few exhibiting relatively large acceleration or deceleration (Figure 3 and Figure 4).

First, we tested whether age acceleration was associated with abusive vs. accidental injuries, without any adjustments. In this model, we observed no significant differences in age acceleration. We then tested multiple adjustment models, including adjusting for sex and insurance status. Again, we observed no significant differences.

Next, we tested whether age acceleration was associated with PRF scores. No significant differences were found in age acceleration when controlling for sex and insurance status.

However, using the PedBE clock, we found that a PRF score of 1, had a statistically significant reduction in age acceleration (p=0.45).

We tested whether injury severity was associated with age acceleration. Using the PedBE clock, we found fatal injuries were associated with an increase in age acceleration. This relationship was true with (p=0.01) and without (p=0.015) adjustments for sex and insurance status.

Next, we tested whether including injury type, PRF scores, and injury severity, revealed a relationship with age acceleration with and without adjusting for sex and insurance status. With adjustments, only injury severity appeared to have a statistically significant increase on age acceleration (p=0.01). Without adjustments PRF score of 1 showed a reduction in age acceleration (p=0.045) and injury severity- fatal showed an increase in age acceleration (p=0.015). Both relationships were found using the PedBE clock.

Given the small data set size of this investigation, we recommend cautionary interpretation of these results. They should be considered exploratory and require independent validation in additional studies.

DISCUSSION

Child maltreatment and neglect is a chronically underfunded public health problem that requires more study in several areas including: how abuse or maltreatment leads to future pathology in the developing child, and ultimately more effective methods of prevention and treatment.

We assessed DNAm profiles in 65 children presenting to a pediatric emergency room ranging in age from 0 to 4 years old using weighted DNAm values at 94 CpG sites (PedBE clock). This model was compared to a model using the 391 CpG DNAm sites in the skin blood

clock. While having a PRF score of 1 was associated with lower age acceleration, all other levels of PRF score exhibited the opposite direction of effect and were not significant. Thus, the potential relationship between age acceleration and psychosocial risk factors was unclear. Overall, we did not observe clear differences in age acceleration, which we had hypothesized may capture one biologically plausible manifestation of physical trauma or psychosocial harm on pediatric biology. We did find that children with higher severity trauma had increased age acceleration when using the PedBE clock. It's unclear whether the physical injuries or the psychosocial environment have a greater impact on age acceleration. However, our findings with injury severity were also driven by a small number of fatal injuries, so it is not clear whether results would be replicated in larger sample sizes.

Shenk et al (2021) found that among children 8-15 years old who had been exposed to maltreatment, epigenetic age acceleration predicted current PTSD diagnosis.[37] Exposure to neighborhood violence is associated with increased age acceleration, though witnessing neighborhood violence was not.[27] In a population of adult women, sexual abuse was associated with increased epigenetic age, while physical abuse and cumulative adversity scores were not.[33] These studies yielded somewhat mixed results. Where age acceleration does appear to be associated with some aspects of adversity in childhood. But it is less clear when the accelerated aging begins, or which specific types of adversity drive these effects. Our results found no relationship between age acceleration and physical abuse or psychosocial risk factors, but we also examined this relationship at one of the youngest ages among published literature. Perhaps, epigenetic age acceleration is a result of cumulative abuse rather than a limited set of occurrences. Also, our comparison group included those children with severe enough accidental injuries to be admitted to the emergency department. It is possible that physical injuries

themselves impact age acceleration at this developmental age, which may have attenuated or masked any differences between our two comparison groups. This would align with prior research on the relationship between neighborhood violence and epigenetic aging. Most studies to date regarding the correlation of maltreatment and epigenetic aging have used blood cells in adults.[38] And these studies use earlier epigenetic clocks, whereas we used the novel PedBE clock, calibrated specifically for buccal cells in children.[26]

We acknowledge the various limits of this study, including the sample size (n=65) and the inherent subjectivity of using experts to categorize physical trauma since these assessments can differ across providers. However, we did use panels of experts, requiring consensus for final determinations. These data are also cross-sectional, so we could not examine whether those with or without abusive injuries exhibited differences in age acceleration in later childhood or adulthood, which would have made for an easier comparison with most published literature. Longitudinal analysis would help to understand how epigenetic age acceleration after these exposures changes over time. With more participants, greater statistical power, wider hospital system participation, and longitudinal data from each participant, further insight would likely be gleaned. Nevertheless, using the PedBE clock, this study adds to the growing evidence of a relationship between a wide array of traumas and increased epigenetic age acceleration in very young children. Further exploration could clarify the effect of psychosocial and physical disturbances on epigenetic aging and more accurately situate our understanding within the wider context of biomarkers of trauma.

REFERENCES

1. Child Maltreatment (2018). Department: U.D.o.H.a.H. Services 2018. https://www.acf.hhs.gov/cb/resource/child-maltreatment-2018
2. Hillis S, Mercy J, Amobi A, Kress H. Global Prevalence of Past-year Violence Against Children: A Systematic Review and Minimum Estimates. *Pediatrics*. Mar 2016;137(3):e20154079. doi:10.1542/peds.2015-4079
3. Jenny C, Hymel KP, Ritzen A, Reinert SE, Hay TC. Analysis of Missed Cases of Abusive Head Trauma. *JAMA*. 1999;281(7):621-626. doi:10.1001/jama.281.7.621
4. Pinheiro PS. *Report of the independent expert for the United Nations study on violence against children* 2006. Accessed 5/29/2020.
5. Leventhal JM, Thomas SA, Rosenfield NS, Markowitz RI. Fractures in young children. Distinguishing child abuse from unintentional injuries. *Am J Dis Child*. Jan 1993;147(1):87-92. doi:10.1001/archpedi.1993.02160250089028
6. Paul AR, Adamo MA. Non-accidental trauma in pediatric patients: a review of epidemiology, pathophysiology, diagnosis and treatment. *Transl Pediatr*. 2014;3(3):195-207. doi:10.3978/j.issn.2224-4336.2014.06.01
7. Bick J, Nelson CA. Early Adverse Experiences and the Developing Brain. *Neuropsychopharmacology*. Jan 2016;41(1):177-96. doi:10.1038/npp.2015.252
8. Norman RE, Byambaa M, De R, Butchart A, Scott J, Vos T. The long-term health consequences of child physical abuse, emotional abuse, and neglect: a systematic review and meta-analysis. *PLoS medicine*. 2012;9(11):e1001349-e1001349. doi:10.1371/journal.pmed.1001349
9. Alexander Butchart APH, Marcellina Mian, Tilman Fürniss, Tony Kahane. *Preventing child maltreatment: a guide to taking action and generating evidence*. 2006.

10. Cohen JR, Menon SV, Shorey RC, Le VD, Temple JR. The distal consequences of physical and emotional neglect in emerging adults: A person-centered, multi-wave, longitudinal study. *Child abuse & neglect*. 2017;63:151-161. doi:10.1016/j.chiabu.2016.11.030

11. Norman RE, Byambaa M, De R, Butchart A, Scott J, Vos T. The long-term health consequences of child physical abuse, emotional abuse, and neglect: a systematic review and meta-analysis. *PLoS Med*. 2012;9(11):e1001349. doi:10.1371/journal.pmed.1001349

12. Gooding HC, Milliren C, Austin SB, Sheridan MA, McLaughlin KA. Exposure to violence in childhood is associated with higher body mass index in adolescence. *Child abuse & neglect*. 2015;50:151-158. doi:10.1016/j.chiabu.2015.08.005

13. McEwen BS, Gianaros PJ. Stress- and allostasis-induced brain plasticity. *Annu Rev Med*. 2011;62:431-45. doi:10.1146/annurev-med-052209-100430

14. McEwen BS, Nasca C, Gray JD. Stress Effects on Neuronal Structure: Hippocampus, Amygdala, and Prefrontal Cortex. *Neuropsychopharmacology*. Jan 2016;41(1):3-23. doi:10.1038/npp.2015.171

15. Sapolsky RM, Krey LC, McEwen BS. Prolonged glucocorticoid exposure reduces hippocampal neuron number: implications for aging. *J Neurosci*. May 1985;5(5):1222-7. doi:10.1523/jneurosci.05-05-01222.1985

16. Baumeister D, Akhtar R, Ciufolini S, Pariante CM, Mondelli V. Childhood trauma and adulthood inflammation: a meta-analysis of peripheral C-reactive protein, interleukin-6 and tumour necrosis factor-α. *Mol Psychiatry*. May 2016;21(5):642-9. doi:10.1038/mp.2015.67

17. Berens AE, Jensen SKG, Nelson CA, 3rd. Biological embedding of childhood adversity: from physiological mechanisms to clinical implications. *BMC Med*. Jul 20 2017;15(1):135. doi:10.1186/s12916-017-0895-4

18. Hertzman C. Putting the concept of biological embedding in historical perspective. *Proc Natl Acad Sci U S A*. Oct 16 2012;109 Suppl 2(Suppl 2):17160-7. doi:10.1073/pnas.1202203109

19. Essex MJ, Boyce WT, Hertzman C, et al. Epigenetic vestiges of early developmental adversity: childhood stress exposure and DNA methylation in adolescence. *Child Dev*. Jan-Feb 2013;84(1):58-75. doi:10.1111/j.1467-8624.2011.01641.x

20. Jang HS, Shin WJ, Lee JE, Do JT. CpG and Non-CpG Methylation in Epigenetic Gene Regulation and Brain Function. *Genes (Basel)*. 2017;8(6):148. doi:10.3390/genes8060148

21. Kramer DA. Commentary: Gene-environment interplay in the context of genetics, epigenetics, and gene expression. *J Am Acad Child Adolesc Psychiatry*. Jan 2005;44(1):19-27. doi:10.1097/01.chi.0000145804.30112.6b

22. Dunn EC, Soare TW, Zhu Y, et al. Sensitive Periods for the Effect of Childhood Adversity on DNA Methylation: Results From a Prospective, Longitudinal Study. *Biol Psychiatry*. May 15 2019;85(10):838-849. doi:10.1016/j.biopsych.2018.12.023

23. Li Z, He Y, Wang D, Tang J, Chen X. Association between childhood trauma and accelerated telomere erosion in adulthood: A meta-analytic study. *J Psychiatr Res*. Oct 2017;93:64-71. doi:10.1016/j.jpsychires.2017.06.002

24. Zannas AS, Arloth J, Carrillo-Roa T, et al. Lifetime stress accelerates epigenetic aging in an urban, African American cohort: relevance of glucocorticoid signaling. *Genome Biology*. 2015/12/17 2015;16(1):266. doi:10.1186/s13059-015-0828-5

25. Horvath S, Raj K. DNA methylation-based biomarkers and the epigenetic clock theory of ageing. *Nat Rev Genet*. Jun 2018;19(6):371-384. doi:10.1038/s41576-018-0004-3

26. McEwen LM, O'Donnell KJ, McGill MG, et al. The PedBE clock accurately estimates DNA methylation age in pediatric buccal cells. *Proceedings of the National Academy of Sciences*. 2019:201820843. doi:10.1073/pnas.1820843116

27. Jovanovic T, Vance LA, Cross D, et al. Exposure to Violence Accelerates Epigenetic Aging in Children. *Scientific Reports*. 2017/08/21 2017;7(1):8962. doi:10.1038/s41598-017-09235-9

28. Cecil CAM, Zhang Y, Nolte T. Childhood maltreatment and DNA methylation: A systematic review. *Neuroscience & Biobehavioral Reviews*. 2020/05/01/ 2020;112:392-409.

29. Reuben A, Moffitt TE, Caspi A, et al. Lest we forget: comparing retrospective and prospective assessments of adverse childhood experiences in the prediction of adult health. *J Child Psychol Psychiatry*. 2016;57(10):1103-1112. doi:10.1111/jcpp.12621

30. Hardt J, Rutter M. Validity of adult retrospective reports of adverse childhood experiences: review of the evidence. *J Child Psychol Psychiatry*. Feb 2004;45(2):260-73. doi:10.1111/j.1469-7610.2004.00218.x

31. Hänninen T, Soininen H. Age-associated memory impairment. Normal aging or warning of dementia? *Drugs Aging*. Dec 1997;11(6):480-9. doi:10.2165/00002512-199711060-00007

32. Pillemer DB, White SH. Childhood events recalled by children and adults. *Adv Child Dev Behav*. 1989;21:297-340. doi:10.1016/s0065-2407(08)60291-8

33. Lawn RB, Anderson EL, Suderman M, et al. Psychosocial adversity and socioeconomic position during childhood and epigenetic age: analysis of two prospective cohort studies. *Human Molecular Genetics*. 2018;27(7):1301-1308. doi:10.1093/hmg/ddy036

34. Lorenz DJ, Pierce MC, Kaczor K, et al. Classifying Injuries in Young Children as Abusive or Accidental: Reliability and Accuracy of an Expert Panel Approach. *J Pediatr*. 2018;198:144-150.e4. doi:10.1016/j.jpeds.2018.01.033

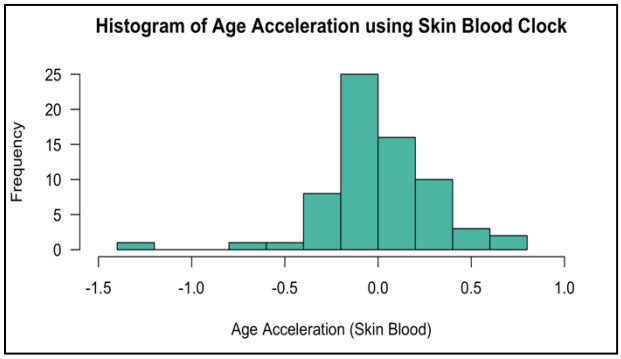
35. Pierce MC, Kaczor K, Acker D, et al. History, injury, and psychosocial risk factor commonalities among cases of fatal and near-fatal physical child abuse. *Child Abuse & Neglect*. 2017/07/01/ 2017;69:263-277.

36. Horvath S, Oshima J, Martin GM, et al. Epigenetic clock for skin and blood cells applied to Hutchinson Gilford Progeria Syndrome and ex vivo studies. *Aging (Albany NY)*. 2018;10(7):1758-1775. doi:10.18632/aging.101508

37. Shenk CE, O'Donnell KJ, Pokhvisneva I, et al. Epigenetic Age Acceleration and Risk for Posttraumatic Stress Disorder following Exposure to Substantiated Child Maltreatment. *Journal of Clinical Child & Adolescent Psychology*. 2022/09/03 2022;51(5):651-661. doi:10.1080/15374416.2020.1864738

38. Gassen NC, Chrousos GP, Binder EB, Zannas AS. Life stress, glucocorticoid signaling, and the aging epigenome: Implications for aging-related diseases. *Neurosci Biobehav Rev*. Mar 2017;74(Pt B):356-365. doi:10.1016/j.neubiorev.2016.06.003

TABLES AND FIGURES



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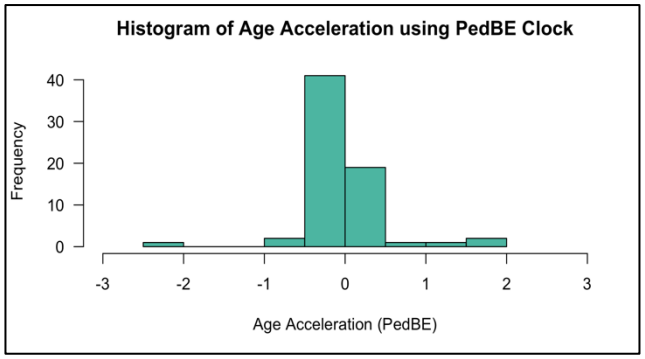

Figure 2

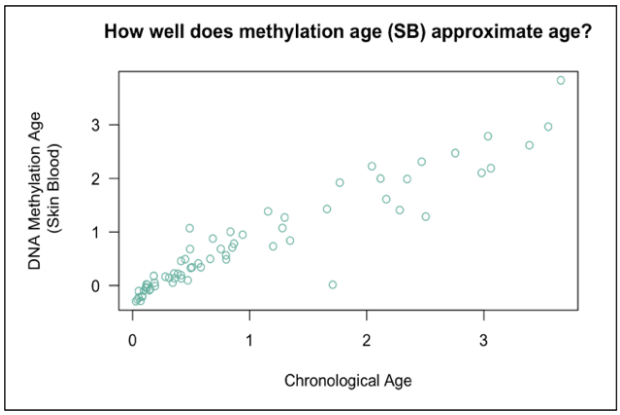

Figure 3

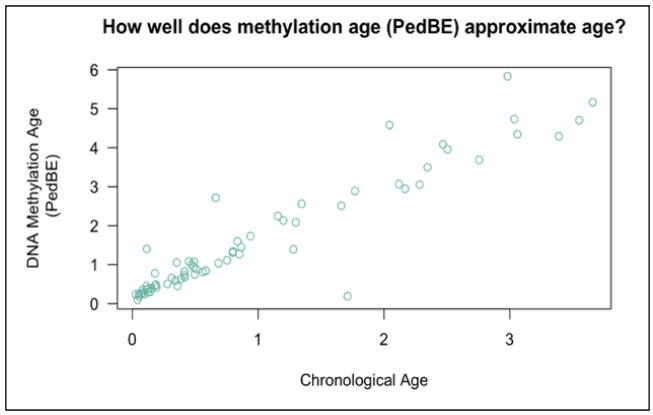

Figure 4